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A DNA-barcoding-based approach to quantitatively investigate larval food resources of cavity-nesting wasps from trap nests

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Abstract

Artificial nesting resources, also known as trap nests, have proven to be an ideal method for monitoring cavity-nesting bees and wasps, their collected food resources, and natural enemies. Nowadays, trap nests are frequently used to assess responses to environmental and biodiversity changes based on multi-trophic interaction networks. Here, we reconstructed quantitative trophic interaction networks of five apoid wasps (*Trypoxylon clavicerum*, *Passaloecus corniger*, *Passaloecus gracilis*, *Psenulus fuscipennis*, *Isodontia mexicana* and two vespid wasp species (*Ancistrocerus nigricornis*, *Microdynerus parvulus*) using DNA barcoding. Sampling the nests during their construction period allowed us to give an accurate count and identification of the provided food items. We recovered highly resolved bi- and tripartite networks including wasp-beetle larva, wasp-cricket, natural enemy-wasp-moth larva, natural enemy-wasp-spider, and natural enemy-wasp-aphid associations. The latter include aphid species that are known as agricultural and forest pests. Although the quantitative sampling of nests entails increased time costs, it enables not only high-quality DNA barcoding but also to reconstruct quantitative interaction networks. Thus, our approach is a highly promising monitoring tool for gaining deeper knowledge on the ecology, habitat requirements and the impact of environmental and biodiversity change on cavity-nesting bees and wasps.

Keywords

Cavity-nesting, DNA barcoding, food resources, tripartite, wasps

Introduction

Bees and wasps play fundamental economic and ecological roles, e.g. as pollinators or to control other arthropod populations including agricultural pest species (Harris 1994; Ollerton et al. 2006; Klein et al. 2007; Fornoff et al. 2023). However, due to diverse anthropogenic factors, bees and wasps are in decline (Senapathi et al. 2015; Hallmann et al. 2017; Trapp et al. 2017; Goulson 2019; Powney et al. 2019; Dicks et al. 2021; Zattara and Aizen 2021). Studying bee or wasp species interactions by means of artificial nesting sites (hereafter referred to as trap nests) is a standardized approach (Staab et al. 2018) to identify and assess environmental drivers associated with population declines. This necessarily includes an in-depth view on trophic interactions, acting bottom-up or top-down on the respective populations, and should be more informative than analyzing co-occurring communities at sampling locations e.g., nesting sites only (Blanchet et al. 2020). Here, we investigate nests of cavity-nesting vespid and apoid wasp species by quantifying their collected food resources as larval provisions, and their natural enemies within a subset of nest cells, to reconstruct highly resolved quantitative multi-trophic interaction networks. The morphological identification of food resources provided to the larvae can be challenging, as insect taxonomist are rare (Hochkirch et al. 2022) or food items can be morphologically unrecognizable, when only parts are left for determination (Fornoff et. al 2023). Thus, we applied DNA barcoding (Turčinavičienė et al. 2016) to overcome these difficulties. In addition, utilizing openable and resealable trap nests allows minimal invasive sampling, collecting fresh material for DNA barcoding and a direct ecological observation of cavity-nesting wasps.

Material and methods

Sample collection

Samples were taken from trap nests set at three different sites in the close surroundings of the University of Hohenheim, Germany from May to August 2022 and 2023 at a weekly base (Suppl. material 5 and Suppl. material 4: table S13). Trap nests are designed to be easily openable and consist of several MDF (medium density fiberboard) boards with ten milled furrows each, covered with a removable acrylic glass, enabling a minimal invasive investigation of a respective nest. Each furrow represents one nest consisting of several nest cells (Fig. 1B–D). Furrows have a diameter of 2.0–9.0 mm to address different sized wasps (Suppl. material 2: table S7). During nest construction and provisioning, a subset of the nest i.e., recently finished nest cells were randomly sampled. Particularly, the whole content of one or a maximum of two nest cells comprising the wasp larva as well as its food provision and potential natural enemies were sampled while leaving remaining nest cells intact. Larvae and food provision were transferred into 100% pure ethanol using sterile forceps and subsequently stored at -20 °C until further processing. Sampling nest cells during nest construction allowed the collection of full-sized and freshly col-

lected prey arthropods before being consumed by the larval wasp. Additionally, one reed stem, also part of the trap nests, was opened and sampled as described before. Please note, in rare cases sampling had to be done for fully constructed nests comprising significantly older larval stages and thus, contained only a few prey individuals or prey remains.

Sample preparation and DNA barcoding

From 19 wasp nests, we sampled a total of 20 nest cells comprising 20 wasp larvae, 361 prey individuals, and six natural enemies (individuals of the natural enemy *Pronotalia* sp. were not counted due to their high number). We separated prey morphotypes under a microscope and selected one specimen per morphotype for the subsequent DNA extraction. Thus, a total of 60 individuals comprising selected prey specimens and wasp larvae were processed for DNA barcoding. We stored the remaining morphotype individuals as voucher specimens.

Genomic DNA (gDNA) was extracted using the nexttec™ 1-Step Tissue & Cells Isolation Kit following the manufactures' protocol with an incubation at 56 °C for 30 min. DNA samples were stored at -20 °C until further processing.

Polymerase chain reactions (PCR) targeting the cytochrome C oxidase I (COI) gene fragment were conducted with the established standard primer pairs HCO2198/LCO1490 (Folmer et al. 1994) and LepF1/LepR1 (Hebert et al. 2003) (sequences in Suppl. material 1: table S1). PCR reactions were set up using the ROTI®Pol TagS Red-Mix in a total reaction volume of 25 µl with 4 or 2 µl template DNA for HCO/LCO and Lepf1/LepR1 reactions respectively (see Suppl. material 1: tables S2, S3). The PCR conditions with HCO/LCO were set as bottom-up reaction starting with 1 min at 94 °C, followed by 15 cycles of 1 min denaturation at 94 °C, 1 min annealing at 40 °C and 1 min elongation at 72 °C and 20 cycles with an annealing temperature of 45 °C and same elongation and denaturation temperatures and times subsequently. The final elongation was set for 5 min at 72 °C. LepF1/LepR1 conditions are included in the supplements (Suppl. material 1: table S5). PCR products were enzymatically purified using the Illustra Exoprostar 1-Step mix, following manufactures protocols and afterwards sequenced on a Sanger-sequencing platform at Microsynth Seqlab GmbH Göttingen, Germany.

Resulting raw DNA sequences were manually edited using Geneious Prime 2023.0.4 (https://www.geneious.com) and searched against the National Center for Biotechnology Information (NCBI) database using the Nucleotide collection (nt/nr) database of the Basic Local Alignment Search Tool BLAST with the following option: highly similar sequences (megablast) (Altschul et al. 1990; Camacho et al. 2009).

Data analysis

The visualization of interactions between the wasp species, their prey and their natural enemies was carried out using R version 4.3.1 (R Core Team 2023) with the R package "bipartite" (Dormann et al. 2008).

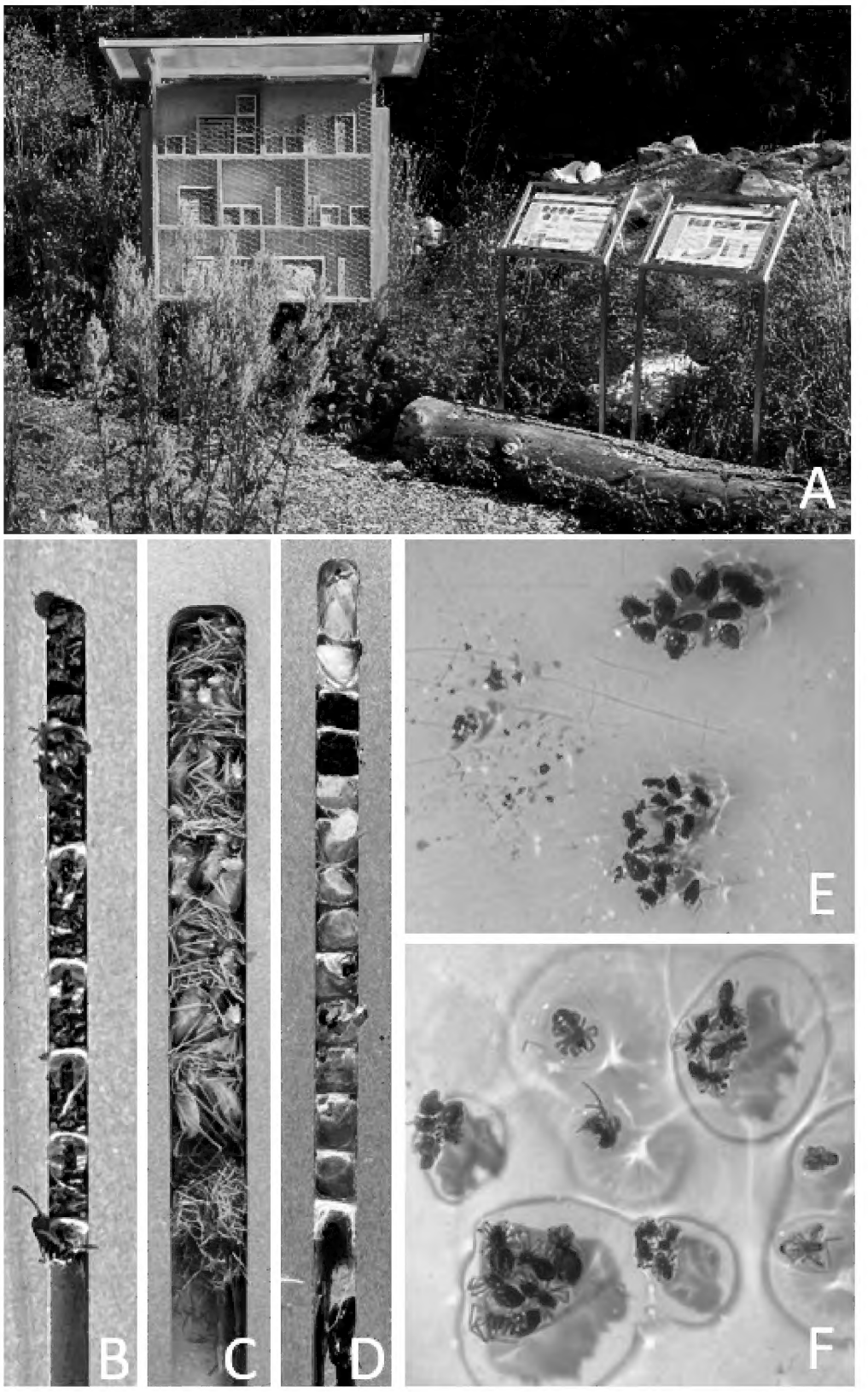

Figure 1. Nesting site and sample collection procedure: **A** example of a trap nest placed in the Botanical Garden of the University of Hohenheim, Stuttgart, Germany **B, C** nests of *Passaloecus gracilis* and *Isodontia mexicana*. One nest comprises several nest cells, which are separated by a given nesting material e.g. silky membran (**B**) or dry grass fragments (**C**) **E** morphotyped aphids **F** morphotyped spiders.

Results

We identified seven species of cavity-nesting wasps comprising one species of the apoid family Crabronidae (*Trypoxylon clavicerum*), two species of Pemphredonidae (*Passaloecus corniger* and *Passaloecus gracilis*), one species of Psenidae (*Psenulus fuscipennis*), one species of Sphecidae (*Isodontia mexicana*) and, two species of vespid wasps belonging to the

family Vespidae (*Ancistrocerus nigricornis* and *Microdynerus parvulus*) (Suppl. material 2: table S6). The latter two species provisioned their larvae with two different species of Lepidoptera (larvae) (Suppl. material 3: table S11) and one species of Coleoptera (larvae) (Suppl. material 3: table S12), respectively. The spider-hunting wasp *T. clavicerum* collected nine different species of Araneae across three different families. Six species of the family Linyphiidae, one species of Araneidae and two species of Tetragnathidae were identified (Suppl. material 3: table S8). The average number of collected prey individuals in *T. clavicerum* nests was 32.25 spiders per nest cell (n = 4, SD = 15.52). Please note, in case only prey remains were available counting nest content was not feasible. The herbivore-hunting wasps *Passaloecus* and *Psenulus* collected nine different species of Aphididae (Suppl. material 3: table S9). The cricket-hunting wasps *Isodontia mexicana* provided its larvae with two species of the genus *Meconema* (Tettigoniidae) (Suppl. material 3: table S10). Furthermore, four species of natural enemies were detected comprising one individual of the parasitoid wasp *Nematopodius* sp., two of the cuckoo-wasp *Trichrysis cyanea* (*P. corniger* and *T. clavicerum*), more than 40 individuals of the chalcid wasp *Pronotalia* sp. (*A. nigricornis*) and two individuals of *Pseudomalus auratus* (*P. gracilis*).

Quantitative multi-trophic networks

The spider-hunting wasp *T. clavicerum* collected the most diverse set of different species as larval food resources (Fig. 2A). Here, each investigated nest cell included at least two but typically three different spider species with varying composition between the nests. Nest cells of the aphid-hunting wasp *P. gracilis* were provided with an average number of 30 aphids (n = 3, SD = 1) comprising only one species: *Aphis ruborum*.

The aphid-hunter *P. fuscipennis* collected five different species of Aphididae, and most of the nest cells contained only a single species (Fig. 2B). The vespid wasp *A. nigricornis* collected eleven Lepidoptera larvae in the investigated nest cell, comprising two different species: *Argyresthia pruniella* and *Hedya pruniana*. The cricket-hunter *I. mexicana* provided eight individuals of the genus *Meconema*, in one nest cell comprising seven of the species *Meconema meridionale* and one *Meconema thalassinum* (Fig. 2C).

Regarding *P. corniger*, nests were sampled several days after provisioning causing prey items to be partly consumed by the larva. Thus, counting collected aphids was not feasible. However, three different species of Aphididae were identified in the larval provisions (Fig. 2B). Furthermore, counting of prey individuals was not feasible for the vespid wasp *M. parvulus*. Here, we identified one larva of the weevil *Tychius picirostris* provided as a larval provision.

Discussion

Quantitative multi-trophic interaction networks provide valuable insights into the feeding ecology of diverse cavity-nesting Hymenoptera and enable conclusions to be drawn about their responses to environmental and biodiversity changes (Staab et

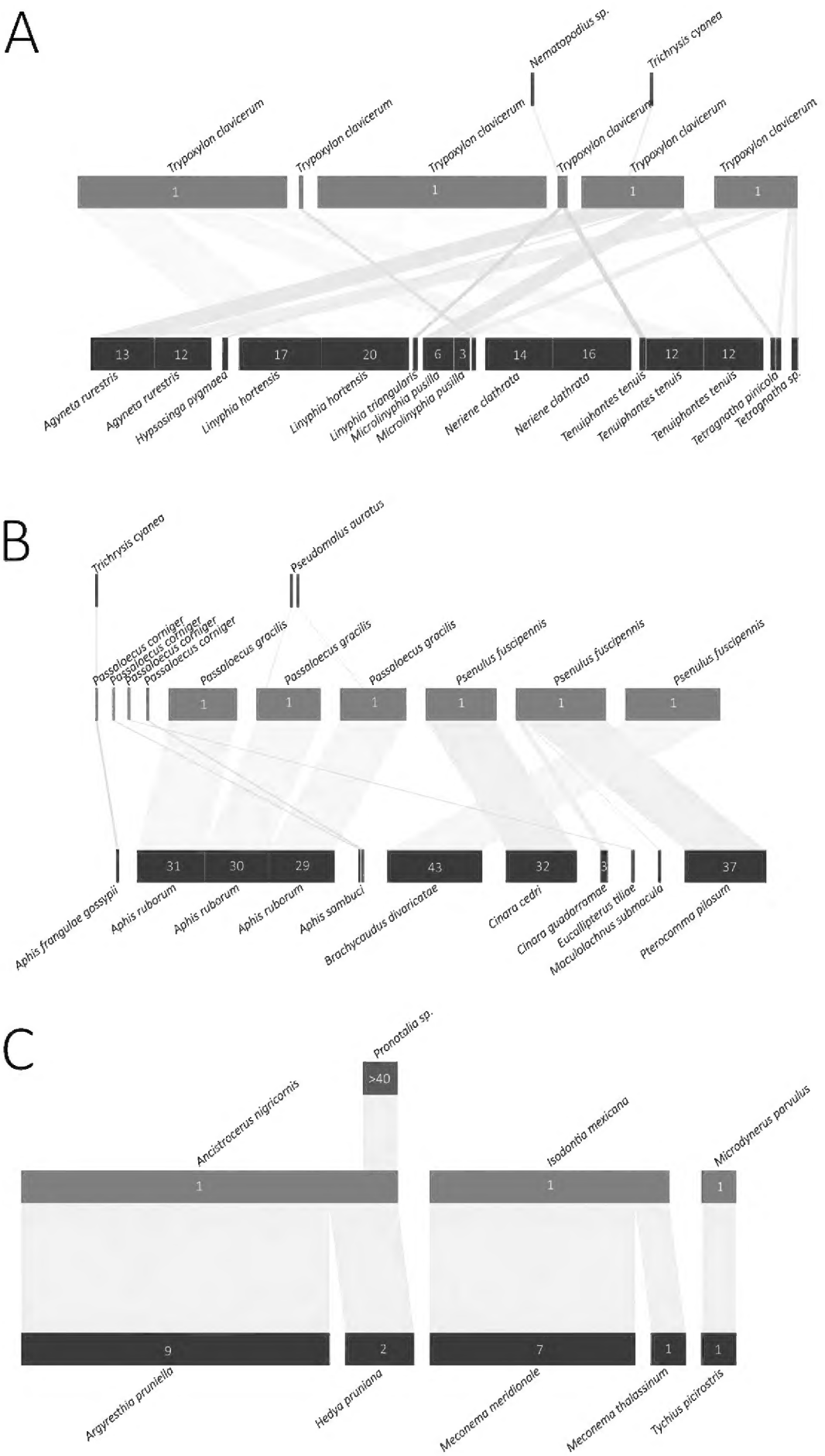

Figure 2. Tri-trophic interaction networks of the studied vespid and apoid wasp species comprising identified prey species and natural enemies. Interaction networks were conducted for the **A** spider-hunting apoid wasp *T. clavicerum* **B** aphid-hunting apoid wasp species *P. corniger*, *P. gracilis* and *P. fuscipennis* and **C** Lepidoptera-hunting vespid wasp *A. nigricornis*, cricket-hunting apoid wasps *I. mexicana* and weevil-hunting vespid wasp *M. parvulus*. Yellow boxes represent the nest cell and the respective wasp larva, blue boxes the natural enemies and green boxes the prey species and the number of prey individuals per species per nest cell. Boxes with no number represent one individual only. The natural enemy *Pronotalia* sp. was not counted due to a high and randomely distributed number of individuals in the nest cell (> 40). Connections of nests and prey species are marked with grey bars.

al. 2018; Fornoff et al. 2023). The here applied minimally-invasive approach allows us to gain comprehensive insides into the larval food provisions of apoid and vespid wasps and increases our knowledge about important feeding links. A major advantage and novelty of the method used here is the quantification of the prey specimens. Linking the quantity and identity of all interaction partners allows e.g., to study individual-based interactions, observe direct feeding links or, interpret the dependence of higher trophic levels on the levels below (Fornoff et al. 2023). Furthermore, this approach facilitates the extraction of high quality and quantity gDNA for subsequent DNA barcoding, given prey items are sampled directly after provisioning. Data analyses requiring higher-quality gDNA also become possible e.g., genetic gut content analyses of prey arthropods. The here presented approach requires a minimum of lab expertise and equipment and thus, might be also interesting for biologist with little or no molecular expertise. In our study, the procedure from sorting morphotypes of prey items to laboratory work to receiving the raw DNA sequence required 15 to 20 minutes hands-on time per sample. However, apart from sorting morphotypes several samples can be processed in the laboratory at the same time. Thus, a common sample size consisting of 96 samples can be prepared for sequencing within one day. Sequencing can be outsourced and requires around 24 hours depending on the sequencing company.

To the best of our knowledge, our approach further allowed the identification of so far unknown or unpublished feeding links: The spider-hunting apoid wasp *T. clavicerum* is known to provide its larvae with spiders of the families Araneidae, Linyphiidae, Tetragnathidae and Dictynidae (Fornoff et al. 2023). Here we expand the family-associated species list given by Fornoff et al. (2023) by five species namely *Hysosinga pygmaea*, *Linyphia hortensis*, *Microlinyphia pusilla*, *Neriena clathrata* and *Tenuiphantes tenuis*. Furthermore, we newly found *Brachycaudus divaricatae* and *Pterocomma pilosum* as host species for the aphid-hunting wasp *P. fuscipennis* as well as *Aphis frangulae gossypii* and *Eucallipterus tiliae* for *P. corniger*. The vespid wasp *A. nigricornis*, known to provide Lepidoptera larvae as larval provision was found to provide larvae of the cherry fruit moth *Argyresthia pruniella* (Argyresthiidae) and the plum tortrix *Hedya pruniana* (Tortricidae). Interestingly, several of the here identified prey species are known as agricultural pests e.g., *A. ruborum* (collected by *P. gracilis*), a potential pest of *Rubus* and *Fragaria* (Alford 2014; Riddick et al. 2019); *C. cedri* (collected by *P. fuscipennis*) a pest on *Cedrus* species (Ji et al. 2021); and *A. pruniella* and *H. pruniana* (collected by *A. nigricornis*), both mainly feeding on trees of the genus *Prunus* (Řezáč 1964). Especially, *A. pruniella* is known to cause high levels of damage to *Prunus* trees, in addition to acting as pest on other orchard crops (Řezáč 1964). Nest cells belonging to the apoid wasps *P. gracilis* and *P. fuscipennis* were mostly found to be filled exclusively with aphids belonging to one species, which probably reflects their agglomerations on the host plant. Given the potential pest risk of some of these aphids, a targeted installation of trap nests might be helpful to control their abundances.

In summary, the combination of standardized trap nest monitoring and DNA barcoding is a useful approach to comprehensively investigate the biology of cavity-nesting Hymenoptera and their interaction partners.

Author contributions

Luisa Timm: Conceptualization, Data curation, formal analysis, investigation, visualization, writing – original draft, Writing – review and editing. Johanna Schaal: Data curation, investigation. Manuela Sann: Conceptualization, Data curation, formal analysis, Funding acquisition, Project administration, Resources, Writing – review and editing.

Data availability statement

Data are deposited as supplementary files. All raw sequencing data are available for download from Mendeley repository: DOI: 10.17632/vjttmbkxpx.1.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Alford DV (2014) Pests of fruit crops: a colour handbook, 2nd ed. Plant protection handbook series. CRC Press-Taylor & Francis (Boca Raton, FL).

Blanchet FG, Cazelles K, Gravel D (2020) Co-occurrence is not evidence of ecological interactions. Ecology Letters 23: 1050–1063. https://doi.org/10.1111/ele.13525

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. BMC Bioinformatics 10: 1–9. https://doi.org/10.1186/1471-2105-10-421

Dicks LV, Breeze TD, Ngo HT, Senapathi D, An J, Aizen MA, Basu P, Buchori D, Galetto L, Garibaldi LA, Gemmill-Herren B, Howlett BG, Imperatriz-Fonseca VL, Johnson SD, Kovács-Hostyánszki A, Kwon YJ, Lattorff HMG, Lungharwo T, Seymour CL, Vanbergen AJ, Potts SG (2021) A global-scale expert assessment of drivers and risks associated with

pollinator decline. Nature Ecology & Evolution 5: 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Dormann CF, Gruber B, Fründ J (2008) Introducing the bipartite package: analysing ecological networks. RNews 8: 8–11.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Fornoff F, Halla W, Geiger S, Klein A-M, Sann M (2023) DNA barcoding resolves quantitative multi-trophic interaction networks and reveals pest species in trap nests. Insect Conservation and Diversity 16(5): 725–731. https://doi.org/10.1111/icad.12664

Goulson D (2019) The insect apocalypse, and why it matters. Current Biology 29: R967–R971. https://doi.org/10.1016/j.cub.2019.06.069

Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D, de Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS ONE 12: e0185809. https://doi.org/10.1371/journal.pone.0185809

Harris AC (1994) *Ancistrocerus gazella* (hymenoptera: Vespoidea: Eumenidae): a potentially useful biological control agent for leafrollers *Planotortrix octo*, *P. excessana*, *Ctenopseustis obliquana*, *C. herana*, and *Epiphyas postvittana* (Lepidoptera: Tortricidae) in New Zealand. New Zealand Journal of Crop and Horticultural Science 22: 235–238. https://doi.org/10.1080/01140671.1994.9513832

Hebert PDN, Cywinska A, Ball SL (2003) Biological identifications through DNA barcodes. Proceeding of the Royal Society London Series 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hochkirch A, Casino A, Penev L, Allen D, Tilley L, Georgiev T, Gospodinov K, Barov B (2022) European red list of insect taxonomists. Luxembourg: Publications Office of the European Union. https://doi.org/10.2779/364246

Ji Y, Li G, Zhou C, Yin S (2021) Influence of temperature on the development and reproduction of *Cinara cedri* (Hemiptera: Aphidoidea: Lachninae). Bulletin of Entomological Research 111: 579–584. https://doi.org/10.1017/S0007485321000419

Klein A-M, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274: 303–313. https://doi.org/10.1098/rspb.2006.3721

Ollerton J, Johnson SD, Hingston AB (2006) Geographical variation in diversity and specificity of pollination systems. In: Waser NM, Ollerton J (Eds) Plant-Pollinator Interactions: from Specialization to Generalization. University of Chicago Press (Chicago), 283–308.

Powney GD, Carvell C, Edwards M, Morris RK, Roy HE, Woodcock BA, Isaac NJB (2019) Widespread losses of pollinating insects in Britain. Nature Communications 10: 1–6. https://doi.org/10.1038/s41467-019-08974-9

R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Řezáč M (1964) Die Schädlichkeit und die Parasiten der auf den mitteleuropäischen Obstbaumarten lebenden *Argyresthia*-Arten und die Möglichkeit ihrer Bekämpfung. Zoologické listy - Folia zoologica 13: 57–72.

Riddick EW, Miller GL, Owen CL, Bauchan GR, Schmidt JM, Gariepy T, Brown, RL, Grodowitz MJ (2019) Discovery of *Aphis ruborum* (Hemiptera: Aphididae) and *Aphelinus varipes* (Hymenoptera: Aphelinidae) on Cultivated Strawberry in Mississippi, USA. Journal of Insect Science 19(3): 17. [1–6] https://doi.org/10.1093/jisesa/iez045

Senapathi D, Carvalheiro LG, Biesmeijer JC, Dodson CA, Evans RL, McKerchar M, Morton RD, Moss ED, Roberts SPM, Kunin WE, Potts SG (2015) The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. Proceedings of the Royal Society B: Biological Sciences 282: 20150294. https://doi.org/10.1098/rspb.2015.0294

Staab M, Pufal G, Tscharntke T, Klein A-M (2018) Trap nests for bees and wasps to analyse trophic interactions in changing environments – a systematic overview and user guide. Methods in Ecology and Evolution 9: 2226–2239. https://doi.org/10.1111/2041-210X.13070

Trapp J, McAfee A, Foster LJ (2017) Genomics, transcriptomics and proteomics: enabling insights into social evolution and disease challenges for managed and wild bees. Molecular Ecology 26: 718–739. https://doi.org/10.1111/mec.13986

Turčinavičienė J, Radzevičiutė R, Budrienė A, Budrys E (2016) Species identification and genetic differentiation of European cavity-nesting wasps (Hymenoptera: Vespidae, Pompilidae, Crabronidae) inferred from DNA barcoding data. Mitochondrial DNA Part A 27: 476–482. https://doi.org/10.3109/19401736.2014.905827

Zattara EE, Aizen MA (2021) Worldwide occurrence records suggest a global decline in bee species richness. One Earth 4: 114–123. https://doi.org/10.1016/j.oneear.2020.12.005

Supplementary material 1

PCR Conditions

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Data type: docx

Explanation note: **table S1.** Primer Sequences; **table S2.** total master-mix and volume for PCR reaction with HCO/LCO primers; **table S3.** total master-mix and volume for PCR reaction with LepF1/LepR1 primers; **table S4.** PCR conditions for HCO/LCO primers; **table S5.** PCR conditions for LepF1/LepR1 primers.

Link: https://doi.org/10.3897/jhr.97.117410.suppl1

Supplementary material 2

Barcode and nest information

Authors: Luisa Timm

Data type: xlsx

Explanation note: **table S6.** Barcode information. Results of BLAST search against the National Center for Biotechnology Information (NCBI) database using the Nucleotide collection (nt/nr) database of the Basic Local Alignment Search Tool BLAST with the following option: highly similar sequences (megablast) (Altschul et al. 1990; Camacho et al. 2009); **table S7.** Nest information.

Link: https://doi.org/10.3897/jhr.97.117410.suppl2

Supplementary material 3

Infos on arthopods

Authors: Luisa Timm

Data type: xlsx

Explanation note: **table S8.** Collected Araneae in nests of *Trypoxylon clavicerum*. Information based on "Spinnen Forum Wiki" > wiki.arages.de <; **table S9.** Collected Aphididae of *Passaloecus corniger*, *Passaloecus gracilis* and *Psenulus fuscipennis*. Information based on Dr. Willem N. Ellis "Leafminers and plant galls of Europe" > https://bladmineerders.nl<, if not differently indicated; **table S10.** Collected Tettigonidae of *Isodontia mexicana*. Information based on J.Fischer et al. "Die Heuschrecken Deutschlands und Nordtirols, Bestimmen - Beobachten - Schützen"; **table S11.** Collected Lepidoptera of *Ancistrocerus nigricornis*. Information based on >https://lepiforum.org<; **table S12.** Collected *Tychius picirostris* of *Microdynerus parvulus*. Information based on Karl Wilhelm Harde, Frantisek Severa und Edwin Möhn: "Der Kosmos Käferführer: Die mitteleuropäischen Käfer".

Link: https://doi.org/10.3897/jhr.97.117410.suppl3

Supplementary material 4

Information on sampling sites
Authors: Luisa Timm
Data type: xlsx
Explanation note: **table S13.** Information on sampling sites at the University of Hohenheim.

Link: https://doi.org/10.3897/jhr.97.117410.suppl4

Supplementary material 5

Trap nests locations
Authors: Luisa Timm, Manuela Sann
Data type: png
Explanation note: supplementary image.

Link: https://doi.org/10.3897/jhr.97.117410.suppl5